

# VU Research Portal

## Balance on many scales: growth and gene expression in *Bacillus subtilis*

Nordholt, N.

2018

### **document version**

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

### **citation for published version (APA)**

Nordholt, N. (2018). *Balance on many scales: growth and gene expression in Bacillus subtilis*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

### **E-mail address:**

[vuresearchportal.ub@vu.nl](mailto:vuresearchportal.ub@vu.nl)

## Summary

Bacterial cells that divide by binary fission double their size and molecular content from birth to division. What sounds like a simple procedure is actually the result of the coordination of countless complex molecular processes that are involved in cell growth and gene expression. With this in mind, it is not surprising that even clonal cells that grow under constant conditions exhibit substantial intercellular differences in their sizes, growth rates and gene expression levels. These phenotypic differences can simply be a consequence of asynchronous growth, i.e. cells having different sizes depending on the time that has elapsed since their birth (their age). They can also arise from the stochastic nature of biochemical processes, i.e. cells having different sizes or molecular compositions at the same age. But despite the complexity of cell growth and gene expression and the randomness that affects them, bacteria are able to maintain homeostasis in cell size and expression levels under constant conditions. This indicates the existence of compensation mechanisms that steer the properties of individual cells, such as size or gene expression levels, towards a condition dependent (optimal) value. The nature of these mechanisms, and the principle cellular properties that they monitor and steer, are not well understood yet. In this thesis, we explore the interrelation and coordination of growth and gene expression in individual bacterial cells by combining theoretical approaches (modelling, microbial growth theory, variance decomposition) with single cell observation techniques (flow cytometry, time-lapse microscopy), using the gram-positive model bacterium *Bacillus subtilis*.

**Chapter 1** provides a brief overview of the history of single cell measurements and introduces key concepts that recur throughout this thesis. In **chapter 2** we extensively review the available literature on the topic of non-genetic heterogeneity in microorganisms and its influence on the ability of a microbial population to survive in and to adapt to dynamic environments.

**Chapter 3** and **chapter 4** constitute the experimental part of this thesis. In **chapter 3** we use flow cytometry to measure the expression levels and variability of a fluorescent protein in individual *B. subtilis* cells under different environmental conditions. We then use theory to decompose and quantify the overall expression variation into variation that arises from different cellular processes. We find that the expression level of the fluorescent protein is the main determinant for the magnitude of expression variability across individual cells and that protein production rate and environmental conditions only have indirect effects on variability, by acting on the expression level. The findings from **chapter 3** can help us in the rational design of genetic circuits that could be of use for production strains in industrial settings. Our results underline the importance of condition dependence for the intended function of a genetic design.

In **chapter 4** we quantify the relationship between growth and gene expression by means of studying the cell cycles of thousands of individual cells, using time-lapse microscopy. We discover systematic and structured deviations from exponential growth along the cell cycle under three independent environmental conditions. We observe a qualitative change in growth behaviour at a fairly constant time to division. Despite the substantial fluctuations that we observe in the growth rates during the life time of each individual cell, we find that gene expression levels stay nearly constant, suggesting that cells compensate these systematic fluctuations. We discuss potential cellular processes that could underlie the observed growth deviations. The results from **chapter 4** might help us to find the mechanisms that decide when cells divide, which leads to cell size homeostasis and allows a bacterial population to reach a state of balanced growth.

Finally, I discuss our experimental findings in the context of recent literature in **chapter 5**. I reflect on the usefulness of phenomenological 'principles' and 'laws' that are used to describe how bacteria are able to maintain cell size homeostasis across many generations. Furthermore, I discuss the question of the evolutionary costs and benefits of non-genetic heterogeneity and gene expression noise. To conclude, I propose experimental approaches that can help us to fill remaining gaps in our

understanding of growth and gene expression in bacterial cells.